Preliminary Amendment US Appl. No.: Not Yet Sssigned Atty. Docket No. 54A-000410US

## **Amendments to the Claims:**

Please amend the claims as shown below. This listing of claims will replace all prior versions and listings of claims in the application.

- 1. (original) A composition comprising an orthogonal leucyl-tRNA (leucyl-O-tRNA), wherein the leucyl O-tRNA comprises an anticodon loop comprising a CU(X)<sub>n</sub> XXXAA sequence, and comprises at least about a 25% suppression activity in presence of a cognate synthetase in response to a selector codon as compared to a control lacking the selector codon.
- 2. (original) The composition of claim 1, wherein the leucyl-O-tRNA comprises a stem region comprising matched base pairs and a conserved discriminator base at position 73 and wherein the selector codon is amber codon.
- 3. (original) The composition of claim 2, wherein the CU(X)<sub>n</sub> XXXAA sequence comprises CUCUAAA sequence and n=0.
- 4. (original) The composition of claim 2, wherein the leucyl-O-tRNA comprises a C:G base pair at position 3:70.
- 5. (original) The composition of claim 1, wherein the leucyl-O-tRNA comprises:
- a first pair selected from the group consisting of: U28:A42, G28:C42 and C28:G42; and,
  - a second pair selected from the group consisting of: G:49:C65 or C49:G65; and, wherein the selector codon is a four-base codon.
- 6. (original) The composition of claim 5, wherein the CU(X)<sub>n</sub> XXXAA sequence comprises a CUUCCUAA sequence and n=1.
- 7. (original) The composition of claim 5, wherein the first pair is C28:G42 and the second pair is C49:G65.
- 8. (original) The composition of claim 1, wherein the CU(X)<sub>n</sub> XXXAA sequence comprises a CUUCAAA sequence and n=0, and wherein the selector codon is an opal codon.
- 9. (original) The composition of claim 1, wherein the leucyl-O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in any one of SEQ ID NO.: 3, 6, 7 or 12, or a complementary polynucleotide sequence thereof.

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- 10. (original) The composition of claim 1, wherein the leucyl-O-tRNA and cognate synthetase, or a conservative variant thereof, are at least 50% as effective at suppressing a selector codon as a leucyl O-tRNA of SEQ ID NO: 3, 6, 7 or 12, in combination with a cognate synthetase.
- 11. (original) The composition of claim 1, further comprising an orthogonal leucyl aminoacyl-tRNA synthetase (leucyl O-RS), wherein the leucyl O-RS preferentially aminoacylates the leucyl-O-tRNA with a selected amino acid.
- 12. (original) The composition of claim 11, wherein the leucyl O-RS, or a portion thereof, is encoded by a polynucleotide sequence as set forth in any one of SEQ ID NO.: 13 or 14, or a complementary polynucleotide sequence thereof.
- 13. (original) The composition of claim 11, wherein the leucyl O-RS comprises an amino acid sequence as set forth in any one of SEQ ID NO.: 15 or 16, or a conservative variation thereof.
- 14. (original) The composition of claim 1, wherein the leucyl-O-tRNA is derived from an archael tRNA.
- 15. (original) The composition of claim 1, wherein the leucyl-O-tRNA is derived from *Halobacterium sp NRC-1*.
- 16. (original) The composition of claim 1, further comprising a translation system.
- 17. (original) A cell comprising a translation system, wherein the translation system comprises:

an orthogonal leucyl-tRNA (leucyl-O-tRNA), wherein the leucyl-O-tRNA comprises at least about a 25% suppression activity in presence of a cognate synthetase in response to a selector codon as compared to a control lacking the selector codon;

an orthogonal aminoacyl-leucyl-tRNA synthetase (leucyl-O-RS); and, a first selected amino acid;

wherein the leucyl O-tRNA comprises an anticodon loop comprising a  $CU(X)_n$  XXXAA sequence and recognizes the first selector codon, and the leucyl O-RS preferentially aminoacylates the leucyl O-tRNA with the first selected amino acid.

18. (original) The cell of claim 17, wherein the leucyl-O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in any one of SEQ ID NO.: 3, 6, 7 or 12, or a complementary polynucleotide sequence thereof, and wherein the leucyl O-RS

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comprises an amino acid sequence as set forth in any one of SEQ ID NO.: 15 or 16, or a conservative variation thereof.

- 19. (original) The cell of claim 17, wherein the leucyl-O-tRNA and cognate synthetase, or a conservative variant thereof, are at least 50% as effective at suppressing a selector codon as a leucyl O-tRNA of SEQ ID NO: 3, 6, 7 or 12, in combination with a cognate synthetase.
- 20. (original) The cell of claim 17, wherein the cell further comprises an additional different O-tRNA/O-RS pair and a second selected amino acid, wherein the O-tRNA recognizes a second selector codon and the O-RS preferentially aminoacylates the O-tRNA with the second selected amino acid.
- 21. (original) The cell of claim 17, wherein the leucyl O-tRNA is derived from *Halobacterium sp NRC-1* and the leucyl O-RS is derived from *Methanobacterium thermoaautotropicum*.
  - 22. (original) The cell of claim 17, wherein the cell is a eukaryotic cell.
  - 23. (original) The cell of claim 17, wherein the cell is a non-eukaryotic cell.
- 24. (original) The cell of claim 23, wherein the non-eukaryotic cell is an *E. coli* cell.
- 25. (original) The cell of claim 17, further comprising a nucleic acid that comprises a polynucleotide that encodes a polypeptide of interest, wherein the polynucleotide comprises or encodes a selector codon that is recognized by the leucyl OtRNA.
  - 26. (original) An E. coli cell comprising:

an orthogonal leucyl-tRNA (leucyl-O-tRNA), wherein the leucyl-O-tRNA comprises at least about a 25% suppression activity in presence of a cognate synthetase in response to a selector codon as compared to a control lacking the selector codon;

an orthogonal leucyl aminoacyl- tRNA synthetase (leucyl-O-RS), wherein the leucyl O-RS preferentially aminoacylates the leucyl O-tRNA with a selected amino acid; the selected amino acid; and,

a nucleic acid that comprises a polynucleotide that encodes a polypeptide of interest, wherein the polynucleotide comprises a selector codon that is recognized by the leucyl O-tRNA, and wherein the leucyl O-tRNA is derived from *Halobacterium sp NRC-1* and the leucyl O-RS is derived from *Methanobacterium thermoaautotropicum*.

(cancelled)